that this is further oxidized by the catalase H_2O_2 system to acetaldehyde. This is not further oxidized by the organism.

I have much pleasure in thanking Sir Patrick Laidlaw for suggesting this work and Prof. Keilin for many helpful suggestions. The work was done during the tenure of the Associate's Research Fellowship, Newnham College.

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INFLUENZA VIRUS ON THE DEVELOPING EGG: VII. THE ANTIBODIES OF EXPERIMENTAL AND HUMAN SERA.

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Received for publication December 15th, 1937.

THERE are three established methods by which antibodies against a given strain of influenza virus can be titrated. These are (1) by the intranasal administration to mice of graded mixtures of serum and mouse-adapted virus (Laidlaw, *et al.*, 1935), (2) by the titration of serum-egg-adapted virus mixtures on the chorio-allantois (Burnet, 1936*b*), and (3) by the complement-fixation test (Smith, 1936; Fairbrother and Hoyle, 1937; Lush and Burnet, 1937). We have already reported (Burnet, Keogh and Lush, 1937) that the results obtained by these three methods do not necessarily correspond. The present communication represents a more detailed study of the problems raised by this lack of correspondence. In view of the great importance which has been attached to antibody titrations of human sera from the point of view of the epidemiology of influenza, it is clearly important that the question of the

* Work supported by a grant for research on virus diseases from the Rockefeller Foundation and the Commonwealth Government Department of Health.

significance of the different types (or manifestations) of antibody should be gone into. In particular the existence of antibody against swine influenza virus in most adult human sera has been interpreted in different fashions by Laidlaw and Shope on the one hand, and Francis on the other. The present work has been done largely in the hope of throwing light on the significance of these swine type antibodies. With the recognition of the sharp antigenic difference between Old World and New World human strains (Burnet, 1937*b*), it became necessary to widen the field of inquiry and use all three antigenic types of virus.

The present paper represents the consolidated experience of two years' work on the virus antibody content of various types of sera. The bulk of the work has been done with the egg membrane technique, but a large number of titrations have been carried out by mouse inoculation methods, and by complement-fixation. In order to present the results as concisely as possible, we have divided the paper into three sections. The first section (A) presents the detailed results of titrating eight sera, chosen to represent each of the main types of serum which had been encountered in the preliminary studies. The sera were quantitatively titrated by each method against each antigenic type of virus. In the second section (B) the antibody content of human sera from various age-groups, as tested by egg membrane titrations against each antigenic type, is described, particular attention being paid to sera from children and adolescents. Finally, in section C we give the results obtained from antibody absorption experiments, which were designed to analyse more closely the characteristic differences between juvenile and adult human sera.

A. COMPARISON OF EIGHT REPRESENTATIVE EXPERIMENTAL AND HUMAN SERA.

The sera of group A were as follows :

1–3. Three ferret immune sera from animals convalescent after infection by " Melbourne " (F. 51), " W. S." (F. 63) and " Swine " (F. 55) strains of virus.

4. The immune horse-serum of Laidlaw *et al.* (1935) (I.H. 2) produced by hyperimmunization of a horse with ferret material infected with strain " W.S."

5. A relatively high titre human serum (K.) from a subject who had a severe attack of presumptive influenza in 1935.

6, 7. Two samples from an adult who showed a well-marked antibody response to the intranasal inoculation of living virus, egg-adapted " Melbourne " strain. The samples R. 1 and R. 2 were taken before and 1 month after inoculation.

8. Serum from a child, aged 4 years, which was known to have a relatively high titre of antibody against " Melbourne ".

Titration methods.—Egg membrane titrations were made as described in previous papers (Burnet, 1936*a* and *b*, 1937*b*), egg-adapted virus of the strains " Melbourne ", " W.S." and " Swine " being used. All sera were heated to 55° C. for 30 minutes soon after they had been separated from the clot, and stored in ampoules or sealed tubes in the refrigerator until used. The only

exception to this rule was the serum I.H. 2, which was received from Dr. C. H. Andrewes about two years previously in liquid form. This was kept in the refrigerator and used without heating. Mixtures were kept in the refrigerator for about 1 hour before being inoculated on fertile eggs at the twelfth day of incubation. After inoculation, eggs were incubated at 36° C. and opened at 40–48 hours in the case of " Melbourne " and " W.S. " strains, and at 66–72 hours for the less active " Swine " strain. The results with the latter strain are less satisfactory than with the two human strains, and a wider margin of error must be allowed in the results. The titre of the serum is expressed primarily as the percentage to which the control count of specific foci is reduced when undiluted serum is added to an equal volume of virus. This percentage is calculated from the experimental results on the basis of two rules which have been fully discussed by Burnet, Keogh and Lush (1937). These are: (1) the " percentage law " of Andrewes and Elford that a given antiserum concentration will reduce the number of foci to a constant percentage irrespective of the absolute concentration of virus present, and (2) the " C.P. = constant " rule that serum concentration × percentage of " surviving " foci will give a constant value. As an example, if a serum diluted 1 : 100 mixed with stock virus reduces the count of foci to 0·1 p.c. of the control, it is assumed that undiluted serum would reduce the count to 0·001 p.c. In many circumstances it is preferable to give a positive figure for the antibody content of a serum, and for this the reciprocal of the percentage reduction is used. In the instance cited, the serum would be given an antibody value of 1000.

Mouse titrations were made by the method of Laidlaw *et al.* (1935). Serial 5-fold dilutions of serum over a suitable range were mixed with equal volumes of undiluted stock virus prepared by grinding finely one consolidated mouse lung with 5 c.c. broth and centrifuging in an angle centrifuge for 10 minutes. The supernatant fluid, which was only slightly opalescent, constituted the stock virus. Three mice were inoculated with each mixture, 0·04 c.c. being given intranasally under anæsthesia. Young mice of 15–20 gm. weight were used. Surviving mice were killed on the sixth or seventh days and the extent of consolidation recorded. The end-point was taken as 40–50 p.c. consolidation of the lungs, and the strength of the serum expressed as 10 times that dilution which, mixed with stock virus, produced an average lesion of this degree. The degree of consolidation was very uniform in mice inoculated with the same mixtures. Where none of the experimental series showed the chosen end-point, a value was interpolated from consideration of the degrees of consolidation recorded.

The complement-fixation tests were carried out as described by Lush and Burnet (1937), and the figure given is the highest final serum dilution which, with optimal antigen concentration, fixed 3 M.H.D. of complement.

The question of using a standard serum and expressing the antibody content of other sera in terms of this has been considered, but, as will be seen when the results are discussed, it is very difficult to decide on what type of serum to use. Actually we have generally used the convalescent ferret sera F. 51, F. 63 and F. 55 as standards for egg membrane and mouse titrations of the corresponding virus strains, and the human serum K as a standard positive serum for complement-fixation tests. In the present work all sera have been

tested in each fashion in a single experiment, and as relative values only are required, the question of an absolute standard does not arise. For convenience, the antibody values are also expressed (Table II) in terms of our sample of serum I.H. 2. This has been preserved as liquid in the refrigerator for two years, and may have deteriorated considerably, but it is the only experimental serum we possess with a wide range of activity against all three virus strains.

TABLE I.—*Titration of Representative Sera by Different Methods.*

Serum.	Egg membrane titrations.			Mouse titrations.			Complement-fixation.	
	Melbourne. %	W.S. %	Swine. %	Melbourne.	W.S.	Swine.	Melbourne.	Swine.
F. 51. Convalescent ferret "Melbourne"	0·001	1·0	22	4000	<10	<1*	100	50
F. 63. „ ferret "W.S."	1·1	0·004	2·4	<20	700	..	100	100
F. 55. „ „ "Swine"	13	9·5	0·0012	2*	..	1000	12·5	50
K. Adult human . . .	0·39	0·2	0·006	220	40	250	100	100
R. 1. „ „ before immunization	5·2	1·0	0·0012	80	20	1000	12·5	12·5
R. 2. Adult human. After immunization with "Melbourne" egg virus	0·15	0·63	0·0012	80	25	1200	12·5	12·5
Ga. Child aged 4 years . .	0·078	10	12	180	<10	<1*	0	0
I.H. 2. Standard horse-serum .	0·077	0·025	0·02	400	140	50	12·5	12·5

* Values derived from results of inoculating undiluted serum + virus diluted 1 : 10.

TABLE II.—*Comparative Activities of the Same Sera in Terms of those of Serum I.H. 2.*

Serum.	Egg membrane titrations.			Mouse titrations.		
	Melbourne.	W.S.	Swine.	Melbourne.	W.S.	Swine.
F. 51 . .	7700	2·5	0·09	1000	<7	<2
F. 63 . .	7	625	0·8	<5	500	..
F. 55 . .	0·5	0·25	1600	0·5	..	2000
K . . .	20	12·5	320	55	28	500
R. 1 . .	1·5	2·5	1600	20	14	2000
R. 2 . .	52	4	1600	20	18	2400
Ga . . .	100	0·25	0·16	45	<7	<2
I.H. 2 . .	100	100	100	100	100	100

An examination of Tables I and II will show a number of points of interest.

1. Neutralization tests with convalescent ferret sera are highly specific, but complement-fixation tests are much less so. These points have been previously discussed (Burnet, 1937*b* ; Lush and Burnet, 1937).

2. Adult human sera show a very high content of antibody capable of neutralizing swine type virus by either method.

3. Normal adult human sera show fairly comparable amounts of antibody against " Melbourne " and " W.S." strains.

4. An adult inoculated intranasally with egg passage " Melbourne " virus shows a sharp rise in antibody detectable by egg membrane titration against the homologous strains, but no significant changes in the antibody determined by other methods of titration.

5. Serum from a young child contains relatively large amounts of neutralizing antibody against " Melbourne " strain, but only insignificant traces against the other two strains.

6. The horse-serum I.H. 2 shows a wide range of activity against the three virus types.

Each of the points 1 to 5 has been found generally applicable to other sera of the corresponding types.

B. THE ACTIVITY OF HUMAN SERA DURING AN INTER-EPIDEMIC PERIOD AGAINST THE THREE ANTIGENIC TYPES OF INFLUENZA VIRUS.

It seemed to us that more information of value would be gained from a detailed examination of small groups of sera from individuals in each main age-group, particularly the adolescent period (10–17), than by accumulation of data from random adult sera.

In Figs. 1 and 2 are represented graphically the antibody titres determined by egg membrane titration of representative sera from the following age-groups :

(1) At birth (umbilical cord blood).
(2) Infants from 3 months to 1 year.
(3) Young children (3–10 years).
(4) Adolescents (11–17 years).
(5) Adults.

Each serum was titrated against each antigenic type, using the egg-adapted strains " Melbourne ", " W.S." and " Swine ". The activity of each serum is expressed logarithmically, using an inverted scale of the percentage to which the count of specific foci is reduced by undiluted serum.

The sera in groups 1, 2 and 4 are random samples. The 4 sera from young children (Group 3) were chosen as those containing the highest antibody titre from a large number which have been tested against " Melbourne " strain on the egg membrane. A majority of young children in Melbourne show no antibody, or much smaller amounts than these do (*see* Fig. 3).

The five adult sera were chosen to provide a sample of each of the main types of serological behaviour which we have encountered in a much larger series of tests.

Taking first the incidence of swine type antibodies, we see the characteristic difference between the activity of sera from young children and from adults, which has been previously described from England and America. Like all other antibodies which have been studied in this respect, influenza virus antibodies of each type pass into the fœtal circulation, but are rapidly lost during the first months of independent life. During childhood those children who have presumably been infected show the appearance of highly specific antibody. Over the transition period 10–16 there are the inevitable irregularities, but there is a clear indication of a gradual increase in swine type antibody over

this period. It is not at all the type of finding one would expect if the swine type antibody were a relic of an infection by " pandemic " influenza, occurring at a very early age, some time between 1920 and 1925.

The serum from the child F. 12, which showed unusually high " Swine " antibody for this age (12 years), was also tested in mice. It was of similarly

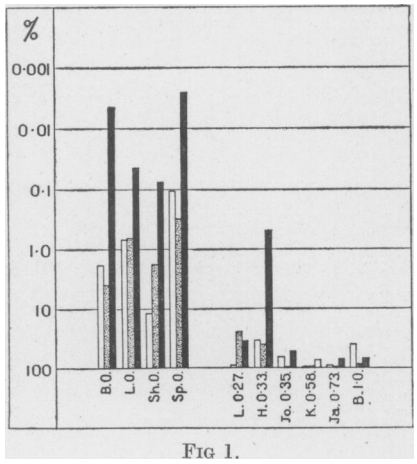

FIG 1.

FIGS. 1 and 2.—Antibody titres of human sera from individuals of various ages as titrated by egg membrane methods against the strains " W.S.", " Melbourne " and " Swine " of influenza virus. The titre is shown logarithmically in terms of the reduction in count of membrane foci using an inverted percentage scale. The vertical columns indicate respectively titres against " W.S." white, " Melbourne " stippled, and " Swine " black for each serum. The sera are arranged in order of age in five groups. The age in years and a distinguishing initial are shown beneath each set of titrations.

FIG. 1.—Sera from umbilical cord blood of newborn infants, and from infants from 3 months to 1 year.

FIG. 2.—Sera from a selected group of young children showing high antibody titres, an unselected series of adolescents from 10 to 17 years, and a small group of adults selected to show different combinations of antibody titres.

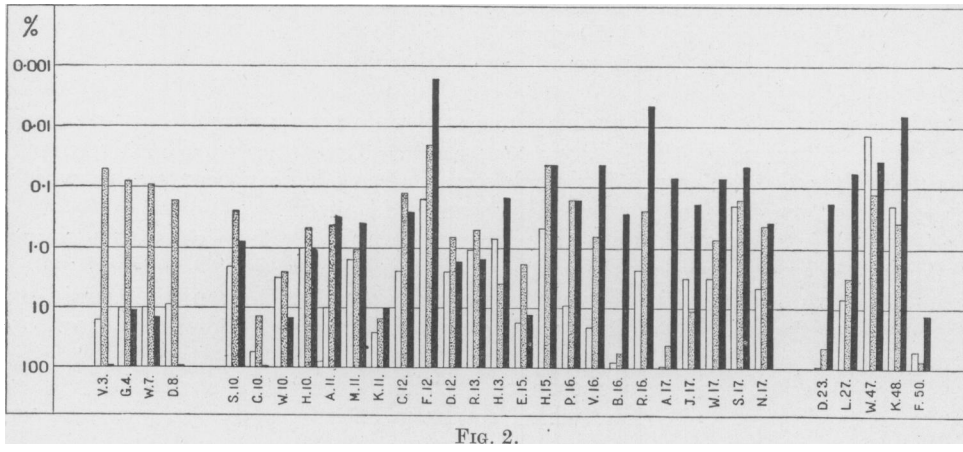

FIG. 2.

high titre, giving a value of 300 according to the convention used in Table I (600 in Table II). As a curiosity it may be noted that the adult subject F., who shows practically no antibody to any type, suffered a typical attack in the 1918 pandemic and has never since had recognizable influenza.

The relative activity against " Melbourne " and " W.S." strains also shows features of interest. In young children who show any antibody it is predominantly against the " Melbourne " strain. In adolescents and adults there is a much closer correlation between the two, " Melbourne " titres being usually

only a little higher than " W.S.". Several subjects, including one 13-year-old, show a significantly higher titre against " W.S." than against " Melbourne ". The obvious interpretation is that the antibody found in the sera of a proportion of young children was induced by infection in 1935 with the " Melbourne " strain then widely prevalent. In previous years epidemics due to various strains, some of the " W.S." antigenic type, must have occurred in Australia, leaving certain individuals with antibody predominantly against this type.

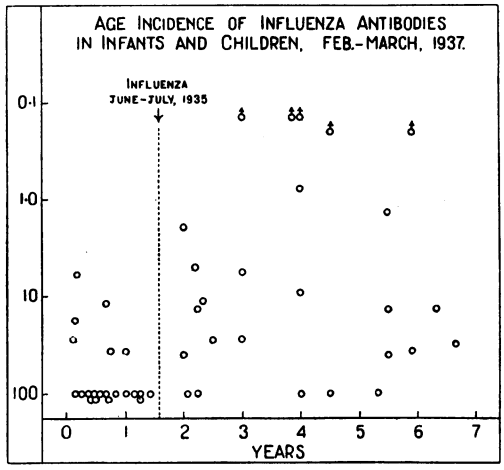

FIG. 3.—Antibody titres against " Melbourne " strain on the egg membrane of sera from infants and children, taken in February and March, 1937, approximately 20 months after an influenza epidemic due to " Melbourne " type virus. Ordinates antibody titre; abscissæ, ages of subjects in years.

In Fig. 3 are shown the results of titrating a large series of sera from infants and young children, taken in February and March, 1937, against " Melbourne " strain. Unfortunately at this period we had not yet adapted " W.S." or " Swine " to egg titrations, so that the information is less complete than it might be. The diagram shows very clearly, however, the relationship between antibody and probable exposure to infection. Of children born since the last appearance of influenza in Melbourne and more than 3 months old, 13 had no trace of antibody, 3 had traces (10–100 p.c.), none any appreciable amount. Of those born before this epidemic, 5 had no trace, 10 traces, 5 medium amounts, and 6 large amounts of antibody.

C. ANTIBODY-ABSORPTION EXPERIMENTS.

We have previously shown that a certain proportion of antibody can be absorbed from convalescent ferret serum by treatment with infected tissues (Burnet, Keogh and Lush, 1937). In general, the antibody demonstrable on the egg membrane is much more readily absorbed than that demonstrable in mouse tests. With some sera it was impossible to demonstrate any absorption of " mouse antibody "; others showed absorption of both types if a large enough absorbing dose were used. This work was done using only the " Melbourne " strain virus and homologous antisera.

In the hope that absorption experiments would throw light on the nature of swine type antibodies, both experimental ferret sera and high titre adult

human sera were absorbed with swine virus (infected mouse lungs) and the absorbed sera titrated. The technique was similar to that previously used. A suitable dilution of serum was prepared, and 2 c.c. added to a ground-up paste of two consolidated lungs. The emulsion was allowed to stand overnight in the refrigerator, centrifuged, and the supernatant fluid heated to 55° C. for 30 minutes. A precipitate usually formed on heating, and this was removed by a final spinning. The supernatant fluid represented the absorbed serum. Many tests have shown that this treatment has no damaging effect on unabsorbed antibodies, and appears actually to protect the antibody against the slight inactivation which occurs when dilute serum is similarly heated. The controls therefore were unabsorbed, and unheated serum diluted to 5/4 the degree of the absorbed serum to allow for the volume of tissue added.

The results obtained are shown in Table III, the degree of absorption being expressed as percentage reduction in antibody activity.

TABLE III.—*Antibody Absorption Experiments.*

Serum.	Absorbed with—	Percentage reduction in antibody activity as tested against—			
		"Swine" (mouse) %	"Swine" (egg) %	"Melbourne" (egg) %	"W.S." (egg) %
F. 55. (1 : 50) Ferret ("Swine")	"Swine" (mouse)	90±	100	..	..
	„ (egg)	75±	90·5	..	..
	"Melbourne" (mouse)	..	*Nil*	..	..
F. 56. (1 : 50) „ „	"Swine" (mouse)	*Nil*	100	..	..
K. (1 : 40) Adult human	„ „	100	100	..	..
K. (1 : 5) „ „	„ „	99±	99·65	81	98·9
W. (1 : 2) „ „	„ „	..	84	91·5	99·3
W. (1 : 5) „ „	„ „	..	99·95	98	99·9
V. (1 : 5) Child aged 3 years	„ „	..	..	*Nil*	..

It will be seen that the ferret sera behave in the same way as homologous ferret sera did with the "Melbourne" strain. F. 56 shows complete dissociation of "egg" and "mouse" antibody, while F. 55 shows considerable reduction by both methods. A heterologous strain "Melbourne" has no absorbing power at all.

The human sera behave quite differently, "Swine" virus being capable of absorbing all types of antibody, *including heterologous activity*, with almost equal readiness. The two human sera tested, K and W, show some differences in behaviour, but there is no doubt at all that very considerable amounts of "Melbourne" and "W.S." antibody are removed. It is probably significant that more activity against "W.S." is removed in each case than against "Melbourne", which was presumably responsible for the clinical infection which both these subjects suffered in 1935.

From the general resemblance between children's sera and those of convalescent ferrets, it would be expected that absorption with "Swine" virus would have no effect in reducing the titre of the specific "Melbourne" antibody. This is found experimentally. Serum V. 3, diluted 1 : 5, was absorbed

according to the usual technique with " Swine " mouse lung virus. No absorption whatever was observed. As is usual in such experiments, the absorbed serum seemed to be slightly, but not significantly, more active than the unabsorbed.

DISCUSSION.

Before discussing the significance of swine influenza antibodies in human sera, we may consider some of the more general points raised by the results shown in Tables I and II.

The changes in the serum of subject R. after intranasal inoculation of living egg-adapted " Melbourne " virus are of particular interest. During the autumn of 1937 about 200 individuals were inoculated in this way with a view to determining whether any protection against clinical influenza could be so afforded. No reports of symptoms which could be ascribed to the inoculations were received, so that it can be taken that the egg-adapted virus has lost its pathogenicity for human beings in the same way as it has for ferrets (Burnet, 1937*a*). As no influenza appeared in Melbourne during the winter, no evidence of protection could be obtained. Eighteen individuals so treated were tested for antibody before and after the inoculation. By the egg membrane titration method about half showed no detectable change, 6 showed slight rises in antibody activity from 2 to 5 times that originally present, and 2, including R., showed an increase to more than 10-fold. Both these sera show the same clear-cut rise in " egg " antibody, and no significant change in the antibody demonstrable by mouse inoculation.

It has been shown previously (Burnet, Keogh and Lush, 1937) that immune ferret sera can be absorbed almost free of antibody demonstrable on the egg membrane, but show no change in the amount of antibody demonstrable in mice. The inference was drawn that only a small proportion of more highly avid antibody molecules were concerned in the egg membrane neutralizations. The results with R. 1 and R. 2 provide confirmation of this point of view from the opposite direction, the active addition of antibody to the serum following immunization causing a large increase in the egg membrane titre, but not influencing the other activities. It is hard to avoid the interpretation that a small amount of " high-grade " specific antibody has been added to the blood, which already contains a much larger number of molecules of " low-grade " antibody of less specific activity.

In this connection we may refer to differences in the ratio between the amount of antibody detectable by egg tests and that found by tests on mice (E/M ratio). Insufficient work has been done with " W.S." in mice to allow conclusions, but with the " Melbourne " strain it has been found regularly that the E/M ratio was much higher for convalescent ferret sera than for normal adult sera or the standard serum I.H. 2. In Table II it will be seen that, taking the E/M ratio for I.H. 2 as unity, that for ferret 51 is 7·7, and for the 2 normal adult sera, 0·36 and 0·075. The last value is an unusually low one. Similarly with " W.S." the E/M ratio for ferret 63 is 1·25, for the normal adult sera 0·45 and 0·18.

This would naturally be interpreted as indicating that convalescent ferret serum contained a much higher proportion of high-grade specific antibody

than normal adult human serum. The child's serum Ga. is intermediate in character, showing an E/M ratio for " Melbourne " of 2·2. This also contains a relatively high proportion of high-grade antibody.

When we examine the E/M ratio for " Swine " virus, we find a significant difference. The ferret serum 55 shows a ratio of 0·8, the adult human sera 0·64 and 0·8, *i. e.* convalescent ferret, adult human and hyperimmunized horse-sera, all show reasonably similar ratios between egg membrane and mouse titrations. In other words, when antibody has any significant action at all on swine type virus, it behaves as if it were of high-grade type.

At one stage we were inclined to regard the antibody persisting in adult human sera during interepidemic periods as of low-grade type more or less equivalent to that remaining in ferret immune sera after absorption with homologous virus. The results of absorption of adult sera with " Swine " virus shown in Table III make it clear that such is not the case. The antibody present is all capable of being absorbed by " Swine " virus, and must be regarded as just as " avid " as the specific antibody of ferret or children's sera, but with a more generalized range of combining power. The absorption experiments at once dispose of any contention that the power to neutralize " Swine " virus is due to antibody produced in response to an infection (during the period 1918–1925, or at any other time) by virus of this specific antigenic type. This is not to say that human beings have never been infected with this type of virus, but merely that the presence of neutralizing antibody supplies no evidence which is relevant to the matter. The evidence put forward by Laidlaw (1935) and by Shope (1936) in favour of the view that such swine type antibodies are a result of infection by the virus responsible for the pandemic of 1918–19 is well known. Their view that " hog flu " first appeared in the American middle West immediately after the pandemic, and was in all probability directly derived from the human infection, appears to be generally accepted.

This in itself, however, does not seem sufficient to justify regarding the human antibodies as having been produced by pandemic type virus. In view of what is known of the rate at which specific antibody titres fall after the antigenic stimulus ceases, it would be extraordinarily unlikely that a child of 12, born in 1925 and infected within its first year or two, should still retain a high content of specific antibody. If any alternative theory is available, it would obviously be preferable.

We consider that the experimental evidence provided in this paper justifies the following description of the development of influenza virus antibodies in an urban population of human beings. The infant at birth possesses the same antibodies as its mother, but loses these within a few months, and shows no antibody against any type of influenza until it is infected by the virus. The antibody produced after infection in childhood is highly specific, showing the same differentiation between the three primary antigenic types of virus as is shown with convalescent ferret sera. During the period of adolescence (10–16 years) there is a gradual appearance of a " generalized " antibody active against all three types, but particularly so against the most readily inactivated type " Swine ". Following infection in adult life, there is a temporary appearance of specific homologous antibody, and if the infection reaches a

clinical level, an increase in the amount of generalized antibody also occurs (Smith and Stuart Harris, 1936). In the absence of fresh clinical or subclinical infection there is a steady diminution of the activity of both specific and generalized antibody, but the activity of the latter against swine antibody remains easily detectable in most subjects throughout adult life.

We may consider the evidence for this hypothesis of the nature of the swine type antibodies in human beings from two points of view, concentrating first on the properties of the antibodies, and second on the nature of the virus concerned. Considering the problem first from the side of the serum, we find that although ferrets, even after hyperimmunization, show very little development of heterologous activity against swine type virus, the horse I.H. 2, also subject to hyperimmunization, shows a considerable degree of such activity. It is well known that different species vary in the specificity of the antibody they produce against a given micro-organism. Topley and Wilson (1936), for instance, mention that human antibodies produced as a result of Salmonella infections are much less specific in their O agglutinins than the selected rabbit sera used for systematic studies of the group. It is reasonable to expect therefore that adult human sera may show a much lower degree of specificity than ferret sera against the influenza viruses. Direct evidence that such is the case may be found from the literature. In 1935 a large number of human sera were examined both by the Hampstead group of workers and by the Rockefeller Institute workers. Despite the fact that they used respectively the strains " W.S." and " P.R. 8 ", now known to be sharply distinct immunologically, there was a general agreement that sera of high titre against one strain were also of high titre against the other.

In our own direct comparative studies of adult sera on this point, there is a general correlation between the titres against the two human types. Careful studies made by Smith and Stuart-Harris (1936) on the serum of a laboratory worker infected with the strain " W.S." showed that along with the rise in titre against " W.S." there was a well-marked increase in the power of the serum to inactivate " Swine " virus.

Finally, our antibody-absorption experiments with adult human sera show conclusively that the same antibody molecules which inactivate human strains of influenza virus are capable of union with swine type virus. The more specific antibody produced after infection in children by strain " Melbourne ", like that produced in ferrets experimentally infected, is not capable of such union.

Turning to consider the question from the side of the virus concerned, we find indications that swine virus differs from the human types in ways which might make it more readily inactivated than these by low-grade antibody. In the first place, swine virus lacks the high degree of lability which is characteristic of human strains, it infects mice and ferrets without adaptation, and retains a relatively constant virulence on passage. Unlike the human strains, it shows only a very low degree of adaptability to the egg membrane. After more than seventy passages it has developed very little more activity than at the beginning, and has no lethal effect on the embryo. It seems to behave as if it were a fixed variant derived by loss of some component or function from some type more closely resembling the current human strains.

Second, the constancy of the E/M ratio (see above) would indicate that with swine virus what is capable of inactivating the virus in the mouse will also inactivate it on the egg membrane. Actually this is not always correct; absorption experiments with the ferret serum F. 56 ("Swine") show that with swine virus in the form of mouse lung almost complete absorption of antibody active on the egg can occur, with no demonstrable reduction in that responsible for inactivation in the mouse. If we use a high titre adult human serum for this experiment, however, both are absorbed to about equal degree.

Third, complement-fixation results show that the optimal concentration of swine antigen is similar for all types of serum and of a type characteristic of the more avid sera against the "Melbourne" strain antigen.

It is obvious that a complete understanding of the immunological reactions of human sera against influenza viruses can be reached only when there has been an opportunity for many years' immunological study of a given population in the light of the strains known to have been responsible for the successive epidemics. In the absence of such experience, interpretation can be based only on the balance of probabilities. In our view, "Swine" influenza antibodies represent "generalized" residual antibody resulting from repeated infections with human type strains. The alternative view, that they represent antibodies derived from infection with "pandemic" influenza, seems almost untenable. It is worth noting, however, that if any laboratory has available any samples of adult human serum taken prior to 1918 it would be possible to provide direct evidence for one or other of the alternatives. Further, if a community which escaped the 1918 pandemic provided sera which contained a normal content of human type antibodies and no swine type antibody, the pandemic theory would be very greatly strengthened. Neither of these more direct types of evidence has yet been provided.

SUMMARY.

1. Representative human and experimental sera have been tested against the three antigenic types of influenza virus by titration on the egg membrane, by intranasal inoculation of mice, and by complement-fixation.

2. A series of human sera from individuals representative of various age-groups has been studied by egg membrane titration of antibody against all three types of influenza virus.

3. The following are the chief results obtained from such titrations:

(i) Experimental ferret sera are highly specific, show a relatively higher titre in egg membrane tests than in mouse titrations, and show some evidence of specificity in complement-fixation tests.

(ii) The immune horse-serum I.H. 2 has a wide range of activity against all three virus types.

(iii) Sera from young children, when they contain antibody, show highly specific reactions, inactivating only the presumed homologous strain "Melbourne" and being without action in swine type virus.

(iv) Adult human sera regularly contain large amounts of antibody capable of neutralizing swine type virus both in mice and on the egg membrane.

(v) Living egg-adapted " Melbourne " virus can be administered by intranasal spray to human volunteers without causing symptoms. In a proportion of individuals so treated, a relatively high rise in antibody was demonstrable by egg membrane titration, but not by mouse tests.

(vi) Human sera at birth contain antibodies against the three types in amounts similar to those of adult sera. Within a few months all antibodies disappear.

(vii) Significant amounts of " Melbourne " type antibody are found in many young children born before the last local epidemic, not in those born since.

4. Absorption of human serum with swine type virus shows characteristic differences between adult and children's sera :

(i) All types of antibody are removed from adult sera.

(ii) Absorption with swine type virus removes none of the specific " Melbourne " antibody from a high titre serum of a child aged 3.

5. These results are discussed, and it is concluded that the swine type antibody in human serum is not specific, but is a manifestation of " generalized " antibody, developing usually about the time of adolescence as a result of repeated infections with human types of virus.

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